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Comparison of Cellular Reporter Systems: GeneBLAzer® and Luciferase

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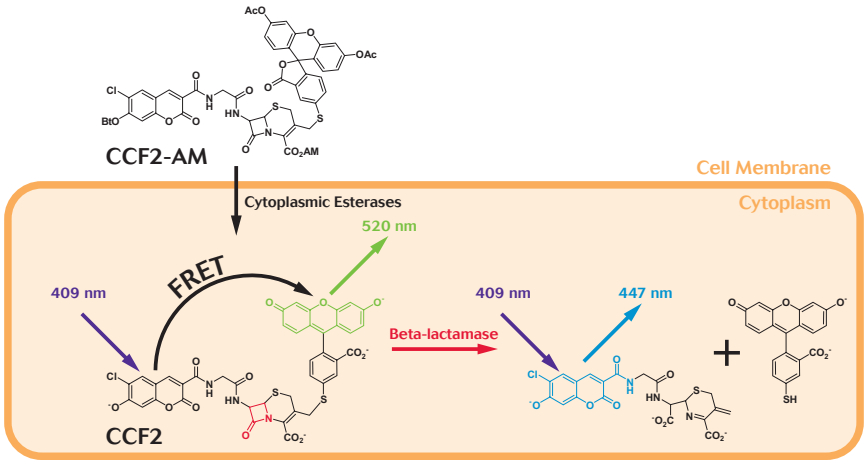
Abstract

Luciferase is a well established reporter gene for cell-based assays; however issues such as the lack of imaging capability, requirement for cell lysis, and a high degree of data variability have made luciferase a less than ideal cellular reporter gene. GeneBLAzer® on the other hand is a cellular reporter system based on the enzyme beta-lactamase and a specialized non-toxic, cell-permeant FRET-paired fluorescent substrate, CCF4-AM. This specialized substrate facilitates imaging and flow cytometry of living cells as well as function-based development of recombinant cell lines. CCF4-AM also allows for ratiometric data analysis, which minimizes the potential impact of fluorescent artifacts and well-to-well variations, without the need for two separate reporter constructs. Overall, GeneBLAzer® is the best gene expression tool available for drug discovery researchers interested in signal transduction pathways, being applicable to a variety of targets and cellular processes including GPCRs, nuclear receptors, kinases, proteases, drug metabolism, and apoptosis.

Introduction

Principles of GeneBLAzer®, the beta-lactamase reporter technology

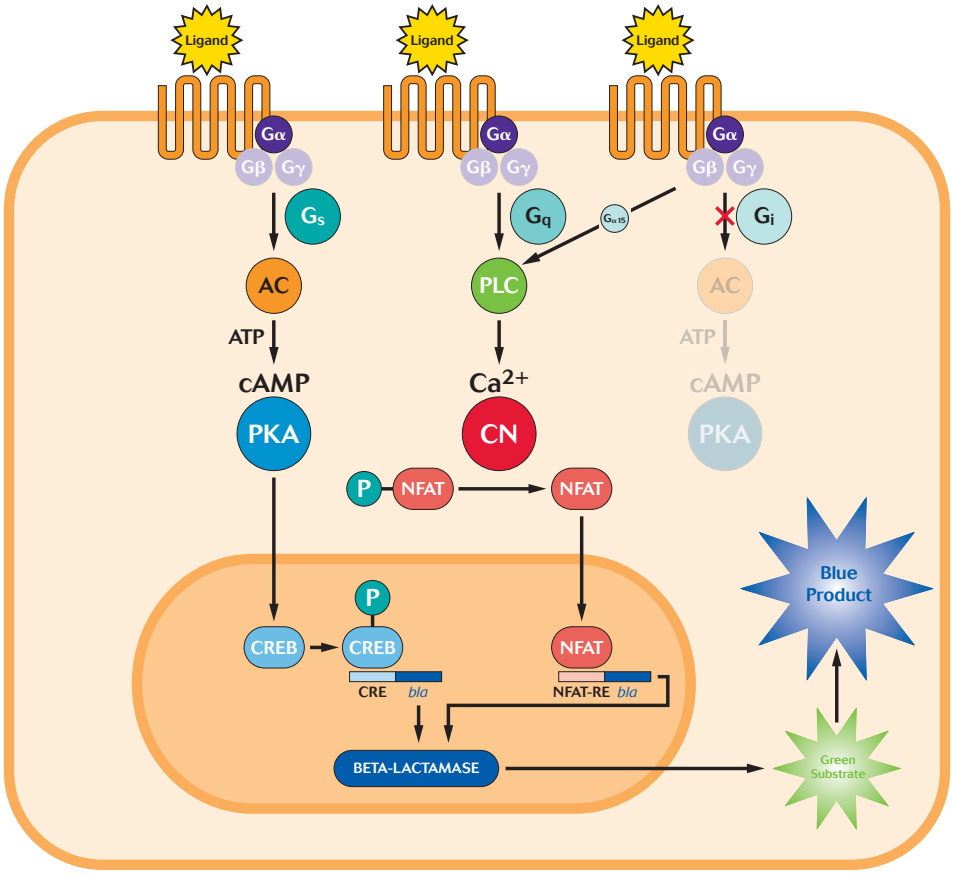
CCF4-AM is a Förster resonance energy transfer (FRET) based substrate for beta-lactamase. Once CCF4-AM enters a cell, it is converted to the negatively charged CCF4 by endogenous esterases. Excitation of this substrate at 409 nm leads to efficient FRET between the coumarin and fluorescein moieties, resulting in a green fluorescence, detectable at 530 nm. Expression of β-lactamase leads to cleavage of CCF4 and results in a loss of FRET, resulting in a robust blue fluorescent signal, detectable at 460 nm.



Introduction, continued

Monitoring of GPCR signaling with GeneBLAzer®

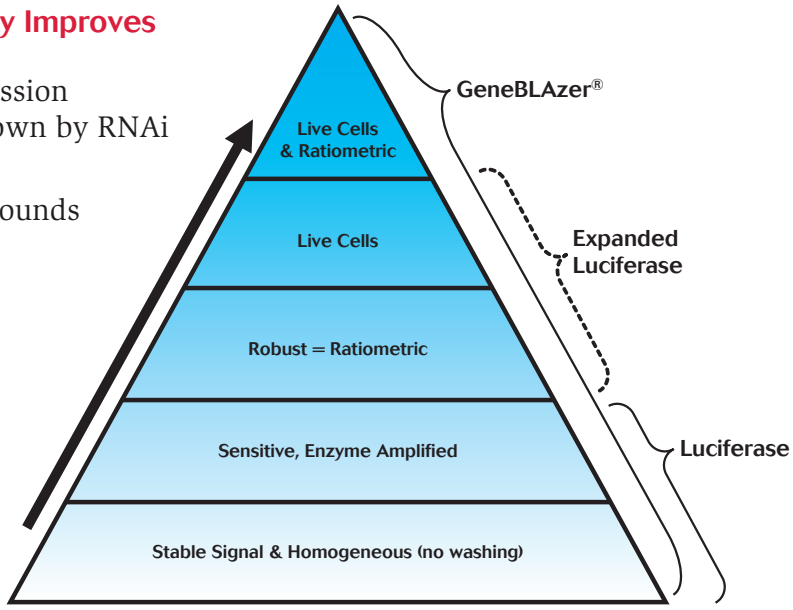
G-protein coupled receptors (GPCRs), regardless of Gα subunit coupling (G_s, G_q, G_i/G_o) can be monitored using beta-lactamase. Stable cell lines expressing the NFAT response element (for monitoring Ca^{2+} flux) or the CREB response element (for monitoring cAMP) linked to the beta-lactamase gene have been developed. These Master cell lines can be used as building blocks to develop target-specific GPCR assays. Gq-coupled receptors can be transfected into the Ca^{2+}-responsive NFAT-*bla* cell line and G_s-coupled receptors can be transfected into the cAMP-responsive CRE-*bla* cell line to follow agonist or antagonist binding events. To track G_i-coupled GPCRs, the promiscuous G-protein, Gα15, can be co-transfected with G_i-coupled receptors into the NFAT responsive cell line to re-direct G_i-coupled signaling to the G_q pathway. Upon stimulation (agonist binding) these cell lines respond with an increase in β-lactamase expression. The β-lactamase response can be quantified using the FRET-based substrate, CCF4-AM, and a standard fluorescence plate reader.



Cellular reporter gene considerations

Assay Quality & Versatility Improves

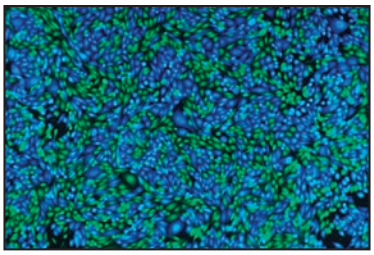
- Detect subtle gene expression
  - Requires less knockdown by RNAi
- Discern toxicity of compounds
- Very tight error bars
  - High Z´-factor values



GeneBLAzer® is the only ratiometric, enzyme-amplified, live cell reporter system

β-lactamase vs. Luciferase

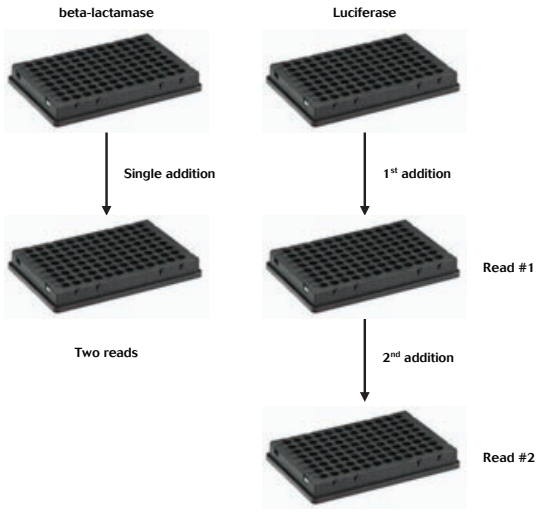
Imaging:



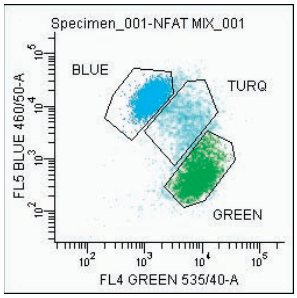
Beta-lactamase = visual data

Luciferase = no imaging

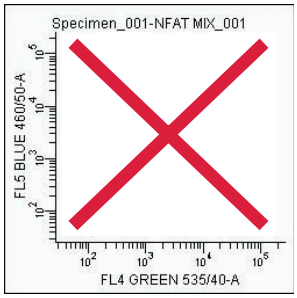
Multiplexing: Reporter gene detection & cell viability



Stable cell line production:

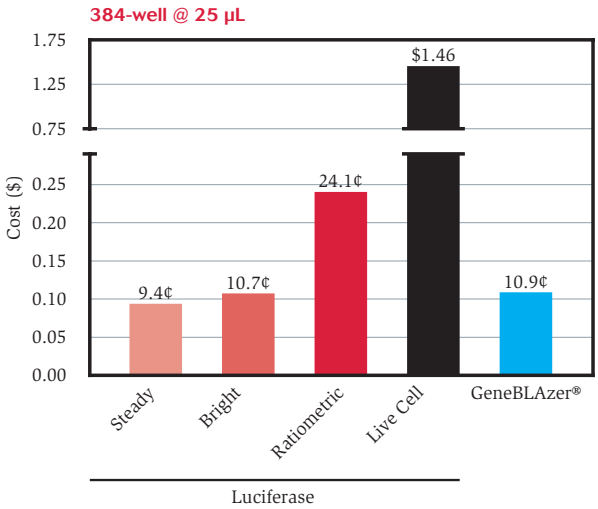


β-lactamase = fast & easy



luciferase = slow & laborious

Cost Comparison for HTS



Ratiometric:

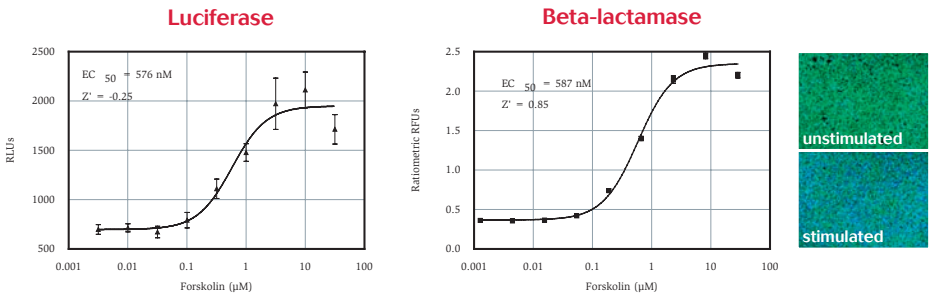
β-lactamase
Simple ratiometric
1 – reporter gene
1 – substrate

Luciferase
Complex ratiometric
2 – reporter genes
2 – substrates

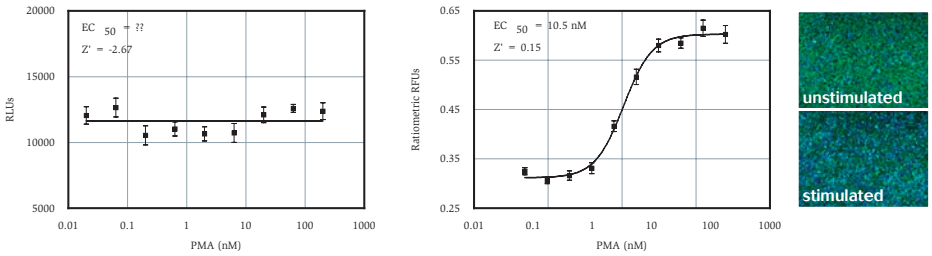
- Average prices based on manufacturers' website list price on June 16, 2004, for the largest unit size available from producers of steady and bright assay kits
- Prices are based on kits (*e.g.*, buffers, substrates, and other additives) not just substrates
- The cost of GeneBLAzer® were computed at 1 µM CCF4-AM, Live Cell at 60 µM EnduRen™

GeneBLAzer® assays vs. Luciferase assays

Adenylyl Cyclase Agonist (cAMP response element)



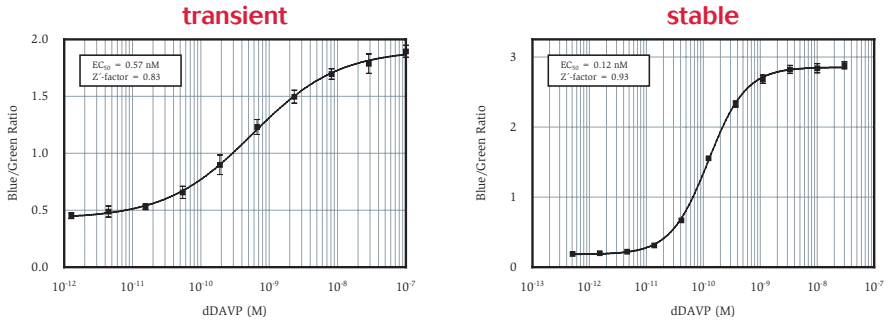
Ca^{2+} release (NFAT response element)



- HEK293 cells transiently transfected with luciferase or beta-lactamase in the same plasmid context
- Steady-Glo® Luciferase Assay System (Promega Corp. Madison, WI) and GeneBLAzer® Loading Kit with CCF4-AM were used to detect luciferase and beta-lactamase, respectively

GPCR Assay Development

Co-expression of CRE-*bla* + Arginine Vasopression GPCR



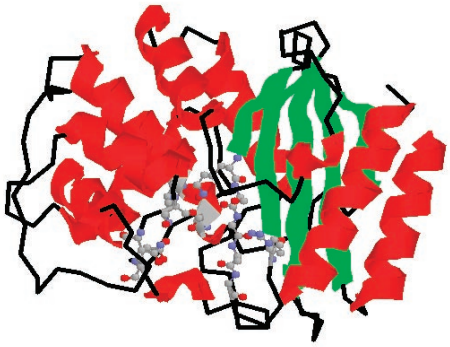
- Similar receptor pharmacology between transient & stable cell lines
- Excellent Z´-factor values for transient & stable cell lines
- Completely homogeneous detection system (performed in complete media with serum and just one addition)

Why is GeneBLAzer® the best cellular reporter system?

- **Simple**—Non-toxic, cell permeable substrate
- **Fast**—Rapid generation of stable cell lines by FACS of live cells
- **Robust**—Ratiometric readout reduces variation
- **Sensitive**—Detection of <100 BLA molecules/cell possible
- **Miniturizable**—Readily scaled down to 3456-well format, ≤ 2 µL
- **Open Architecture**—No license fees
- **Support**—Complete product offerings, including custom assay development

Small bacterial enzyme
~29 kDa

Simple kinetics:
$k_{cat} = 29 \pm 1\ s^{-1}$
$K_m = 23 \pm 1\ \mu M$



No mammalian
Background

Highly efficient
$k_{cat}/K_m = 1.3$

Half-life in cells
5 hours

No cofactors required for activity

Monomeric

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